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**EP 0 258 067 B1**

## Description

This invention relates to the field of recombinant DNA technology. In particular it relates to a novel yeast promoter.

Recombinant DNA technology makes it possible to express heterologous gene products in *Saccharomyces cerevisiae*. This is achieved by constructing gene fusions between appropriate non-coding regulatory DNA sequences and the DNA sequence encoding the structural protein to be expressed. In this respect the 5' and 3' non-coding regions of the phosphoglycerate kinase (PGK) gene of *S. cerevisiae* have been used to construct expression vectors capable of synthesizing commercially important polypeptides in yeast (Tuite et. al., 1982; Kingsman & Kingsman, 1982; Mellor et. al., 1983). However, although these vectors are able to direct the synthesis of significant quantities of heterologous polypeptides in yeast, they are subject to the same physiological factors which influence the expression of the native yeast protein.

Thus, for example, when cells are grown in a medium supplemented with a fermentable carbon source, such as glucose, PGK promoter-directed expression is 20-30 fold higher than that observed when cells are grown in a medium containing a non-fermentable carbon source (Tuite et. al., 1982). This regulation of gene expression is mediated at the level of DNA transcription (Holland & Holland, 1978) and can be attributed to the properties of the 5' non-coding region of the PGK gene. This 5' non-coding region can be divided into functional domains which have similar properties to those observed in other 5' non-coding regions of yeast genes. In particular, a DNA sequence has been identified between nucleotides -324 and -445 which has the ability to activate DNA transcription when located upstream of a yeast 5' non-coding region (Kingsman & Kingsman, 1984). This DNA sequence, referred to as the PGK upstream activation sequence (UAS) is essential for the transcriptional activation of the PGK gene (Kingsman & Kingsman, 1984).

An analogous situation exists for the activation of other yeast genes. For example *GAL1* and *GAL10* of *S. cerevisiae* are activated by an UAS which confers galactose regulation of gene transcription (Guarente et. al., 1982; Johnston & Davis, 1984). This UAS we refer to as the *GAL10* UAS. The *GAL10* UAS can also be located upstream of other yeast gene 5' non-coding regions where it confers galactose regulation on DNA transcription (Guarente et. al., 1982).

The PGK 5' non-coding region is generally considered to be an extremely strong yeast promoter capable of mediating high level gene expression under optimal physiological conditions, i.e.

when cells are grown in the presence of a fermentable carbon source. Whilst PGK expression can be regulated by a judicious choice of carbon source, expression is not subject to absolute control since significant levels of gene transcription occur in the presence of non-fermentable substrates. Consequently, the PGK promoter can not be used for the efficient regulation of heterologous gene expression in yeast. WO 84/04757 discusses the PGK promoter and its UAS.

Moreover, the PGK promoter is inappropriate for application within the context of European Patent Application No: 86303039.1, published under No 0201239. This application relates to the production of ethanol and a heterologous protein or peptide by fermenting an aqueous carbohydrate-containing medium with a yeast such as brewer's yeast which has been genetically modified to be capable of expressing a heterologous protein or peptide under conditions such that the yeast multiplies but no expression of the protein or peptide occurs, recovering the ethanol so formed, inducing expression of the protein or peptide by the yeast and obtaining the protein or peptide therefrom. Galactose regulation of expression of the heterologous gene is particularly useful here, since the medium in which the yeast is grown, brewers wort, does not normally possess sufficient galactose to induce the transcriptional activation and thus expression of galactose-regulated genes. Furthermore, genes which normally are regulated by galactose exhibit a high degree of inducible control. Thus, for example, when cells are either resuspended and/or grown in a medium supplemented with galactose, galactose-regulated transcription is approximately 1000-fold higher than that observed in the absence of galactose (Hopper et. al., 1978; St. John & Davis, 1979). This high level of induction is in contrast to that described earlier for PGK promoter-directed expression, which is only 20-30 fold inducible. However, whilst galactose-regulated gene expression enables a high degree of inducible gene regulation, it does not necessarily result in a concomitant high level of gene expression under fully induced conditions. EP-A-164 556 discloses certain hybrid promoters.

We now provide a hybrid yeast promoter comprising components of the 5' non coding region of the PGK gene and regulatory components from the *GAL10* UAS. This has the advantage of conferring galactose regulation of gene transcription upon a modified 5' non-coding region' of the inherently efficiently expressed PGK yeast gene. This results in the formation of a hybrid promoter which confers high level transcriptional activity in the presence of galactose, that is, under fully induced conditions, but low level (barely detectable) activity in the absence of galactose. Thus the hybrid promoter

possesses the transcriptional activity of the PGK gene and the regulatory properties of the galactose regulated gene.

The new hybrid promoter comprises the GAL10 UAS fused to a modified 5' non-coding region sequence of the PGK gene and does not incorporate the endogenous PGK UAS found, in the natural PGK promoter, between nucleotide coordinates -423 and -470. It is preferred that the GAL10 UAS is provided at the deletion site of the PGK UAS. The GAL10 UAS may be present in either orientation.

The hybrid promoter may be prepared by inserting the GAL10 UAS into a suitable site in the 5' non-coding region of the PGK gene. The 144 base-pair Rsa I - Alu I DNA fragment derivable from the GAL1-GAL10 promoter may be inserted.

Yeast expression vectors, typically plasmids, incorporate the hybrid promoter to control the expression of heterologous or homologous proteins or peptides. A wide range of heterologous proteins or peptides may be expressed. By way of example, mention may be made of enzymes such as beta-lactamase, beta-glucanase and beta-galactosidase. Other useful heterologous proteins and peptides include materials of human origin and/or useful in therapy, such as human serum albumin and immunoglobulins.

An expression vector may be constructed by inserting in a vector incorporating the hybrid promoter a gene coding for the protein or peptide which it is desired to express. The gene can be inserted at a restriction site which is provided downstream of the translational start codon controlled by the hybrid promoter. The gene must be inserted in the correct translational reading frame. A fusion product containing the protein or peptide of interest will then be expressed. Alternatively, the gene may itself be provided with a translational start codon followed directly by a DNA sequence encoding the protein or peptide of interest. Such a gene may be inserted in a vector incorporating the hybrid promoter but which does not incorporate a translational start codon. In such a vector, a restriction site is so provided that into the site the gene may be inserted in the correct reading frame and such that its translational start codon is correctly positioned in relation to the hybrid promoter. The expression vector is provided with a transcription terminator sequence. This may be the PGK terminator sequence.

The expression vectors can be used to direct the galactose-regulated high level expression of genes in a transformant yeast. The vectors may be used to transform laboratory strains of *Saccharomyces cerevisiae*. They may be used to transform industrial strains of *Saccharomyces cerevisiae* such as the top fermenting ale yeasts

(*S. cerevisiae*) and the bottom fermenting lager yeasts (*S. uvarum* or *S. carlsbergensis*). The expression vectors are particularly useful for transforming brewer's yeast and can be used to provide galactose regulation of the process for the production of heterologous proteins and peptides according to European Application No. 86303039.1 as described above.

A peptide or protein may be obtained from the transformed yeast by growing and/or placing the yeast in a galactose-containing medium to switch on high level expression of the peptide or protein. Thus, a peptide or protein may be prepared by growing and/or placing in a galactose-containing medium a yeast which has been transformed by a yeast expression vector in which the expression of the said peptide or protein is controlled by a hybrid promoter of the invention and obtaining the said peptide or protein thus produced.

The following Example illustrates the invention. In the accompanying drawings:

Figure 1 shows the location of GAL10 UAS insertions in the 5' non-coding region of the PGK gene;

Figure 2 is a diagrammatic representation of the divergent GAL1-GAL10 promoter region of *S. cerevisiae*;

Figure 3 illustrates the construction of plasmids pDB1 and pDB2;

Figure 4 shows the Bal 31 deletion series of pDB4;

Figure 5 shows the 5' non-coding region of pDB4 and the hybrid PGK-GAL UAS promoter constructs.

## EXAMPLE

### Materials and Methods

#### Strains, media and transformations

Strains used were *E. coli* AKEC 28 (C600, thrC, leuB6, thyA, trpC117, hsdR<sub>k</sub>, hsdM<sub>k</sub>) and *S. cerevisiae* DBY745 ( $\alpha$ , ura3-52, ade1-100, leu2-3, leu2-112).

*E. coli* cultures were grown on LB medium (Miller, 1972) supplemented where appropriate with the antibiotic ampicillin (Sigma Chemical Co. Ltd., Poole, Dorset, England.) at 50  $\mu$ g/ml final concentration. Yeast were grown at 30 °C on a synthetic complete medium (SC) (0.67% w/v yeast nitrogen base without amino acids) supplemented with carbon source and amino acids where appropriate.

*E. coli* was transformed using standard methods (Maniatis, et. al., 1982). Yeast was transformed as described by Hinnen et. al., (1978).

### Recombinant DNA techniques

Standard procedures were used for restriction endonuclease digestion and the construction of plasmid DNA (Maniatis et. al., 1982). All enzymes were obtained from Bethesda Research Laboratories (Paisley, Scotland) and were used according to the manufacturers recommendations. Exonuclease Bal 31 was used for the in-vitro deletion of DNA sequences as described by Dobson et. al., (1982). Deletion end-points were determined by DNA sequencing (Sanger et.al., 1977; Maxam & Gilbert, 1980). BglII synthetic oligonucleotide linkers were obtained from Collaborative Research Inc. (Lexington, Massachusetts, USA).

### DNA & RNA Isolation

Plasmid DNA was isolated from *E. coli* by the methods of Chinault and Carbon (1979) and Birnboim and Doly (1979). The method of Holmes & Quigley (1981) was used for the rapid analysis of plasmid DNA. Total yeast DNA was prepared according to Cryer et. al., (1975). Total RNA was prepared from yeast cells grown to a density of  $4 \times 10^6$  cells ml<sup>-1</sup> as described previously (Dobson et. al., 1982).

### Hybridisation and DNA probes

Northern and Southern transfers were performed using standard procedures (Maniatis et. al., 1982). Hybridisation of <sup>32</sup>PdTTP (Amersham International Ltd., Amersham) nick translated (Rigby et. al., 1977) DNA probes was performed according to Thomas (1980). PGK (Mellor et. al., 1983), Ty (Dobson et. al., 1984) and rDNA (Petes et. al., 1978) DNA probes were labelled to a specific activity of  $4.6 \times 10^7$  cpm/μg DNA following purification from agarose gels (Tabak & Flavell, 1978).

### Determination of plasmid copy number and RNA analysis

Total yeast DNA was digested with the restriction endonuclease EcoRI and separated by electrophoresis in a 1% w/v agarose gel. DNA fragments were transferred to nitrocellulose and hybridized to radioactively labelled PGK and rDNA specific DNA probes to estimate plasmid copy number. Regions of DNA homology were highlighted by autoradiography. By comparing the relative intensity of the rDNA and PGK specific regions of homology it was possible to estimate the number of copies of the PGK specific DNA sequence. This was facilitated by the knowledge that there are approximately 100-140 repeats of the genomic rDNA per haploid genome (Pertes et. al., 1978).

This method of plasmid copy number determination is generally applicable providing that an appropriate plasmid DNA probe is utilized in the assay.

Total RNA was separated by electrophoresis in 1% w/v agarose containing 6% w/v formaldehyde. RNA was transferred to nitrocellulose filters as described previously and hybridized with nick translated DNA probes. A transposon Ty mRNA species of 5700 nucleotides or a ribosomal DNA probe of 1800 nucleotides was used as an internal loading control in hybridizations to enable a direct comparison between different transformants.

### Results

#### Analysis of the 5' non-coding region of the PGK gene

A series of deletion 'windows' have been constructed in the 5' non-coding region of the yeast PGK gene (Fig.1). These were obtained by ligating a combination of DNA fragments possessing deletions in to the 5' non-coding region of the PGK gene from both the 5' and the 3' direction. 5' to 3' deletions were obtained in a derivative of plasmid pMA27 (Mellor et. al., 1983) in which the Cla I site at position -800 in the PGK 5' non-coding region had first been converted to a unique Xho I restriction site using a synthetic oligonucleotide linker. This pMA27 derivative was then cleaved with Xho I and digested with Bal 31 exonuclease. Plasmids were recircularized by ligation in the presence of Bam HI synthetic oligonucleotide linkers and transformed into *E. coli*. Plasmid DNA was isolated and the positions of the 3' deletion end-points were characterised by DNA sequencing. 3' to 5' deletions were obtained in plasmid pMA22a (Dobson et. al., 1982), following cleavage at the unique Bam HI site and Bal 31 exonuclease digestion. Plasmids were recircularized by ligation in the presence of Bam HI synthetic oligonucleotide linkers and transformed into *E. coli*. Plasmid DNA was isolated and the positions of the 5' deletion end-points were similarly characterized by DNA sequencing. Plasmids pDB3, pDB4 and pDB5 (Fig.1) were then constructed by ligating Bam HI-Pst I fragments containing appropriate combinations of the 5' and 3' deletion derivatives. Thus DNA sequences downstream of the 3' end-points were obtained from the 5' deletion derivatives, whereas DNA sequences upstream of the 5' end-points were obtained from the 3' deletion derivatives.

Plasmids pDB3 and pDB5 possess deletions which are located 5' and 3' of the PGK UAS respectively, whereas pDB4 has a deletion between coordinates -423 and -470 which includes the PGK UAS itself (see figs. 1 and 5). Each of the aforementioned plasmids was constructed such that a

unique Bam HI restriction site bounded the deletion end-points. This facilitates subsequent DNA insertions.

Yeast transformed with plasmids pMA27, pDB3 and pDB5 produces comparable high levels of PGK mRNA when grown on SC supplemented with various carbon sources, whereas yeast harbouring plasmid pDB4 produces levels of PGK mRNA equivalent to those produced by the untransformed yeast. This indicates that the PGK UAS is essential for PGK mRNA synthesis.

#### Construction of a galactose inducible PGK based gene

The organization of the GAL1-GAL10 divergent promoter of yeast is shown schematically in Figure 2. The functional region of the UAS has been localized (West et. al., 1984) and its position is indicated along with flanking restriction sites (Fig. 2). A 365 base-pair DNA fragment is to be found on plasmid pLGSD5 (Guarente et. al., 1982) which carries the GAL10 UAS. The 144 base-pair Rsa I-Alu I DNA fragment from the GAL1-GAL10 promoter region on pLGSD5 was purified from a polyacrylamide gel and blunt-end ligated into the unique Sma I site of pUC8 (Fig.3). Subsequently, the unique Eco RI site of pDB1 (Fig.3) was converted to a Bgl II site by the insertion of a synthetic Bgl II oligonucleotide linker. Thus the 144 base-pair GAL10 UAS could be isolated on a unique Bgl II-Bam HI DNA fragment carried by plasmid pDB2 (Fig.3). This fragment was subsequently cloned into the unique Bam HI site in each of the three PGK deletion vectors pDB3, pDB4 and pDB5; GAL10 UAS inserts were obtained in either orientation to derive plasmids designated pKV41-pKV46 (Figure 1).

Plasmids pKV43, pKV44, pMA27 and pDB4 were transformed into strain DBY745 and the levels of PGK specific mRNA were determined during exponential growth on media containing either glucose or galactose as carbon source. The results indicated, in the case of pKV43 and pKV44 that PGK specific mRNA could be induced to high levels in the presence of galactose, whereas growth on glucose resulted in chromosomal levels of PGK specific mRNA. Transformants harbouring plasmid pMA27 showed high levels of PGK specific mRNA when grown on both glucose and galactose medium, however pDB4 showed no activity on either carbon source. These results clearly demonstrate that the replacement of the PGK UAS with the GAL10 UAS, in either orientation, confers high level galactose regulated DNA transcription on the PGK promoter. Transformants harbouring plasmids pDB3 and pDB5 maintained high levels of PGK specific mRNA on both glucose and galactose me-

dium, comparable to that from plasmid pMA27. In the case of plasmids pKV41, pKV42, pKV45 and pKV46 high levels of PGK specific mRNA are maintained on both carbon sources. These results show that it is not sufficient to insert the GAL UAS at any site 5' of the transcription initiation sequence of PGK in order to confer galactose regulation upon transcription, but rather it is necessary both to insert the GAL UAS and to remove the PGK UAS. Thus in the case of pKV43 and pKV44 the PGK UAS has been deleted and replaced by the GAL UAS.

#### Construction of a galactose regulated PGK expression vector

Plasmid pDB4 was digested at a unique Bgl II site located in the 3' region of the PGK structural gene and the linear molecule was digested with Bal 31 exonuclease (Fig.4). DNA fragments were filled-in and re-ligated in the presence of excess Bgl II synthetic oligonucleotide linkers; plasmids thus formed were screened by gel electrophoresis and DNA sequencing to determine the precise 'end-point' of the deletion. A series of deletion derivatives were obtained differing in the nucleotide sequence immediately 5' of the Bgl II linker. Deletion derivatives with 'end-points' at positions -8 (pKV47), +4 (pKV51), +5 (pKV52) and +6 (pKV53) were obtained (Fig.4).

The deletion derivatives were further modified by the attachment of the 3' transcription terminator sequence of the PGK gene. This was accomplished by digesting each plasmid with the restriction endonucleases Bgl II and Pst I and ligating the large fragment thus generated with the small Bam HI-Pst I fragment containing the 3' transcription terminator sequence of the PGK gene derived from plasmid pMA91 (Mellor et. al., 1983). Plasmids thus formed were then further modified by the insertion at the unique Bam HI site in the modified 5' non-coding region of the PGK gene of the Bgl II-Bam HI fragment containing the GAL 10-UAS from pDB2 (fig 3.). The orientation of insertion of the GAL 10-UAS was then determined by restriction enzyme digestion analysis to produce plasmids indicated in figure 5.

In this manner a series of galactose regulated PGK expression vectors were obtained in which the PGK-UAS had been replaced by the GAL10 UAS. The DNA sequence surrounding position 1 of the PGK coding region for each of these vectors is depicted in Figure 4. Whereas, the DNA sequence of the modified PGK 5' non-coding region into which the GAL10 UAS has been inserted is presented in Figure 5.

Expression vectors pKV49 and pKV50 (Figs. 4 and 5) can be used to mediate the expression of

heterologous and homologous genes in which the 5' translational initiation signal (ATG) is supplied by the gene of interest. Under circumstances in which the gene to be expressed does not possess a 5' translational initiation signal (ATG), translational fusion vectors can be used. In this respect deletions in the PGK coding sequence ending at positions +4, +5, and +6 (fig. 4) facilitate fusion of the gene to be expressed into each of the three possible reading frames. These deletion derivatives have been used in the construction of expression vectors pKV61-66. Plasmids pKV61 and pKV62, pKV63 and pKV64, pKV65 and pKV66, are analogous to pKV49 and pKV50 respectively in respect of the orientation of the GAL10 UAS (Fig 5).

#### The expression of human serum albumin in yeast

A cDNA clone encoding the human serum protein albumin (HSA) was isolated on a 1.84 kilobase-pair BamHI DNA fragment from plasmid pEK113 (described in European Patent Publication No. 0201239A in the name of Delta Biotechnology Ltd.) and subcloned into the unique Bgl II site of the expression vectors pKV49 and pKV50 (figs. 4 and 5) to generate plasmids pKV59 and pKV60 respectively (fig. 6). This HSA encoding DNA sequence had previously been manipulated to include a 5' translational initiation signal immediately adjacent to the first codon of the mature HSA coding sequence (European Patent Publication No. 0201239A). The DNA sequence at the 5' junction of the HSA gene with pKV49 and pKV50 is indicated in Fig.7.

Plasmids pKV59 and pKV60 were transformed into the laboratory yeast strain DBY745 by standard procedures. Transformants were subsequently grown on SC supplemented with adenine and uracil plus either glucose (1% w/v) and galactose (1% w/v) or galactose (1% w/v), representing repressing and inducing carbon sources respectively. Cultures were harvested at a cell density of  $4-6 \times 10^6$  per ml and used to prepare extracts of total DNA, total RNA and protein. The results presented in fig. 8 clearly show that galactose induces the synthesis of HSA specific mRNA, whereas in the presence of glucose little or no HSA specific mRNA can be detected. Cell extracts were also assayed for HSA protein following SDS:polyacrylamide gel electrophoresis and Western blotting (European Patent Publication No. 0201239A). The results of these gels were consistent with the mRNA analyses described above since substantial quantities of HSA could be detected in yeast grown in the presence of galactose, whereas yeast grown in the presence of glucose produced much lower, but detectable quantities of HSA. When protein bands on SDS:polyacrylamide gels were visualized following

coomassie blue staining a significant proportion of the total cellular protein was constituted by the HSA protein in the galactose induced cultures, whereas no HSA band could be visualized in cultures grown in the presence of glucose.

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## Claims

1. A hybrid yeast promoter comprising, as an upstream activation sequence, the upstream activation sequence of the GAL10 gene of Saccharomyces cerevisiae and, as a downstream portion, a downstream portion of an S. cerevisiae promoter effective in-promoting transcription of a coding sequence placed downstream thereof, characterised in that the said S. cerevisiae promoter is that of the PGK gene and the hybrid promoter does not contain the endogenous PGK upstream activation sequence located, ii the natural PGK promoter, between nucleotide coordinates -423 and -470.
2. A hybrid yeast promoter according to claim 1, in which the GAL10 upstream activation sequence is provided at the site from which the PGK upstream activation sequence has been deleted.
3. A yeast expression vector including a hybrid promoter as claimed in any one of the preceding claims.
4. A yeast expression vector according to claim 3, in which a restriction site has been provided downstream of the translational start codon controlled by the hybrid yeast promoter to enable a gene which it is desired to express to be inserted thereinto in the correct translational reading frame.
5. A yeast expression vector according to claim 3, in which
  - (a) no translational start codon is provided at the translational start site controlled by the hybrid yeast promoter but
  - (b) a restriction site is provided into which a gene, which it is desired to express and which possesses a translational start codon, may be inserted such that the translational start codon of the gene is in the correct position in relation to the hybrid yeast promoter.

6. A yeast expression vector according to claim 3, in which a homologous gene which it is desired to express is provided under the control of the hybrid yeast promoter.

7. A yeast expression vector according to claim 3, in which a heterologous gene which it is desired to express is provided under the control of the hybrid yeast promoter.

8. A yeast expression vector according to claim 7, in which the heterologous gene encodes human serum albumin.

9. A yeast transformed with a vector as claimed in any one of claims 3 to 8.

10. A yeast according to claim 9, which is a transformed brewing yeast.

11. A process for preparing a peptide or protein, which process comprises maintaining in a galactose-containing medium a yeast which has been transformed by a yeast expression vector in which the expression of the said peptide or protein is controlled by a hybrid promoter as claimed in claim 1 or 2 and obtaining the said peptide or protein thus produced.

## Patentansprüche

1. Hybridhefepromotor, umfassend, als Stromaufwärtsaktivierungssequenz, die Stromaufwärtsaktivierungssequenz des GAL10-Gens von Saccharomyces cerevisiae und, als Stromabwärtsteil, einen Stromabwärtsteil eines S. cerevisiae-Promotors mit der Wirksamkeit zur Förderung einer Transkription einer stromabwärts davon befindlichen Codiersequenz, dadurch gekennzeichnet, daß der S. cerevisiae-Promotor derjenige des PGK-Gens ist und der Hybridpromotor die endogene PGK-Stromaufwärtsaktivierungssequenz, die bei dem natürlichen PGK-Promotor zwischen den Nukleotidkoordinaten -423 und 470 lokalisiert ist, nicht enthält.
2. Hybridhefepromotor nach Anspruch 1, wobei die GAL10-Stromaufwärtsaktivierungssequenz an der Stelle, an welcher die PGK-Stromaufwärtsaktivierungssequenz deletiert wurde, vorgesehen ist.
3. Hefeexpressionsvektor, enthaltend einen Hybridpromotor nach einem der vorhergehenden Ansprüche.

4. Hefeexpressionsvektor nach Anspruch 3, wobei eine Restriktionsstelle stromabwärts des durch den Hybridhefepromotor gesteuerten Translationsbeginncodons vorgesehen ist, um ein zu exprimierendes Gen in diese in dem korrekten Translationsleserahmen insertieren zu können. 5
5. Hefeexpressionsvektor nach Anspruch 3, bei welchem
  - a) an der durch den Hybridhefepromotor gesteuerten Translationsbeginnstelle kein Translationsbeginncodon vorgesehen ist, statt dessen 10
  - b) eine Restriktionsstelle vorgesehen ist, in die ein zu exprimierendes Gen mit einem Translationsbeginncodon derart insertiert werden kann, daß das Translationsbeginncodon des Gens in korrekter Lage in Bezug auf den Hybridhefepromotor liegt. 15
6. Hefeexpressionsvektor nach Anspruch 3, in dem unter Steuerung des Hybridhefepromotors ein zu exprimierendes homologes Gen vorgesehen ist. 20
7. Hefeexpressionsvektor nach Anspruch 3, in dem unter Steuerung des Hybridhefepromotors ein zu exprimierendes heterologes Gen vorgesehen ist. 25
8. Hefeexpressionsvektor nach Anspruch 7, in dem das heterologe Gen Humanserum Albumin codiert. 30
9. Hefe, die mit einem Vektor nach einem der Ansprüche 3 bis 8 transformiert ist. 35
10. Hefe nach Anspruch 9, bei der es sich um transformierte Brauereihefe handelt. 40
11. Verfahren zur Herstellung eines Peptids oder Proteins durch Halten einer Hefe, die durch einen Hefeexpressionsvektor, in dem die Expression des Peptids oder Proteins durch einen Hybridpromotor nach Anspruch 1 oder 2 gesteuert wird, transformiert wurde, in einem galactosehaltigen Medium und Gewinnen des erhaltenen Peptids oder Proteins. 45

#### Revendications 50

1. Promoteur de levure hybride comprenant, comme séquence d'activation amont, la séquence d'activation amont du gène *GAL10* de *Saccharomyces cerevisiae* et, comme portion avale, une portion avale d'un promoteur de *Saccharomyces cerevisiae* efficace pour promouvoir la transcription d'une séquence co-

dante placée en aval, caractérisé en ce que ledit promoteur de *Saccharomyces cerevisiae* est celui du gène PGK et en ce que le promoteur hybride ne contient pas la séquence d'activation amont de PGK endogène située, chez le promoteur PGK naturel, entre les nucléotides de coordonnées -423 et -470.

2. Promoteur de levure hybride selon la revendication 1, dans lequel la séquence d'activation amont de *GAL10* est fournie sur le site à partir duquel a été retirée la séquence d'activation amont de PGK.
3. Vecteur d'expression de levure comprenant un promoteur hybride tel que revendiqué dans l'une quelconque des revendications précédentes.
4. Vecteur d'expression de levure selon la revendication 3, dans lequel un site de restriction est fourni en aval du codon d'initiation de translation contrôlé par le promoteur de levure hybride de sorte qu'un gène que l'on désire exprimer puisse y être inséré dans le cadre de lecture de translation approprié.
5. Vecteur d'expression de levure selon la revendication 3, dans lequel
  - a) aucun codon d'initiation de translation n'est fourni sur le site d'initiation de translation contrôlé par le promoteur de levure hybride mais
  - b) dans lequel un site de restriction est fourni, dans lequel un gène, que l'on désire exprimer et qui possède un codon d'initiation de translation, peut être inséré de sorte que le codon d'initiation de translation du gène se trouve dans la position appropriée par rapport au promoteur de levure hybride.
6. Vecteur d'expression de levure selon la revendication 3, dans lequel un gène homologue que l'on désire exprimer est fourni, sous le contrôle du promoteur de levure hybride.
7. Vecteur d'expression de levure selon la revendication 3, dans lequel un gène hétérologue que l'on désire exprimer est fourni, sous le contrôle du promoteur de levure hybride.
8. Vecteur d'expression de levure selon la revendication 7, dans lequel le gène hétérologue code pour l'albumine sérique humaine.
9. Levure transformée avec un vecteur tel que revendiqué dans l'une quelconque des revendications 3 à 8.

10. Levure selon la revendication 9, qui est une levure de bière transformée.

11. Procédé de préparation d'un peptide ou d'une protéine, lequel comprend le maintien dans un milieu contenant du galactose, d'une levure ayant été transformée par un vecteur d'expression de levure, dans lequel l'expression dudit peptide ou de ladite protéine est contrôlée par un promoteur hybride tel que revendiqué dans la revendication 1 ou 2, et l'obtention dudit peptide ou de ladite protéine ainsi produits.

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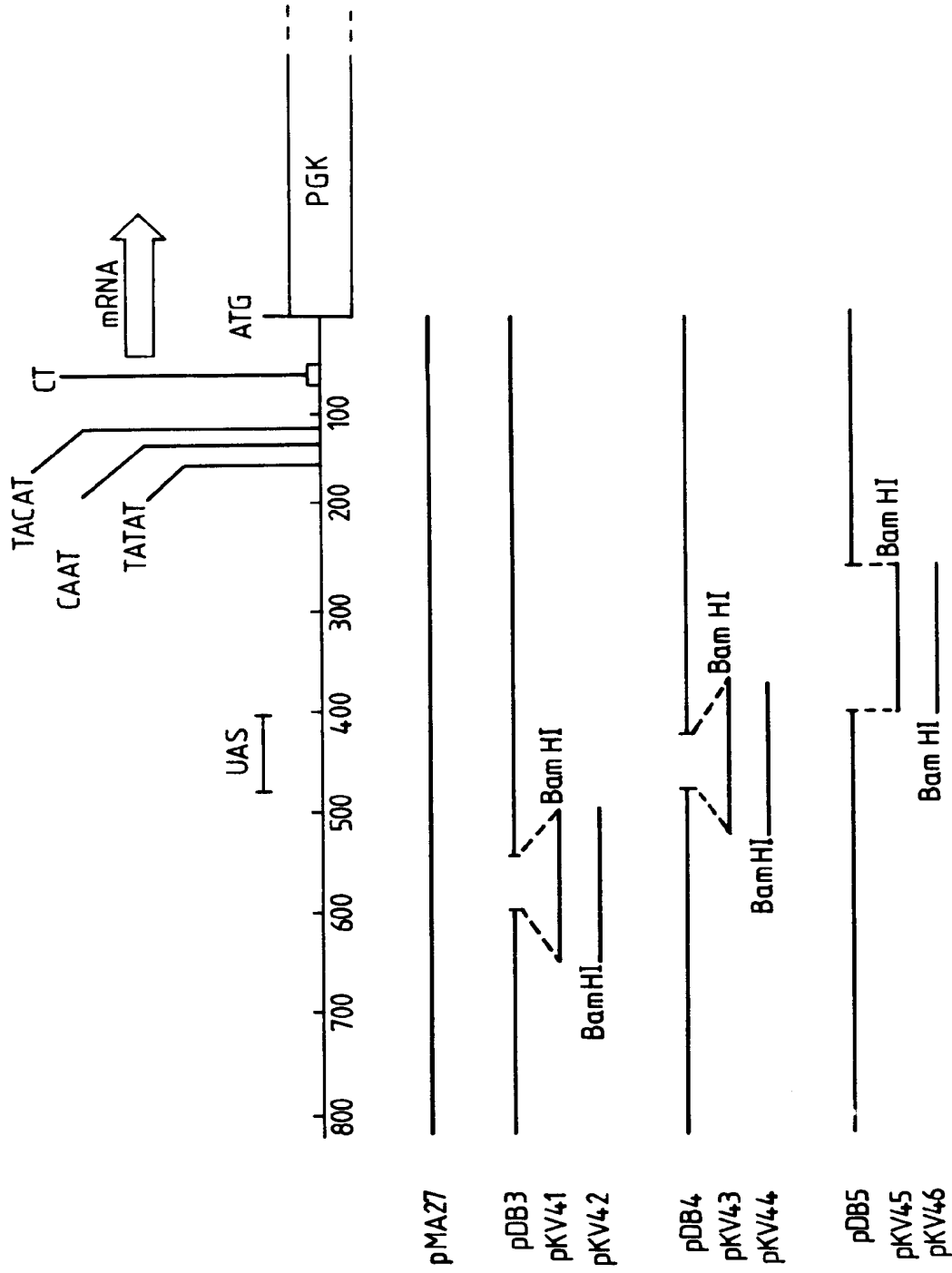
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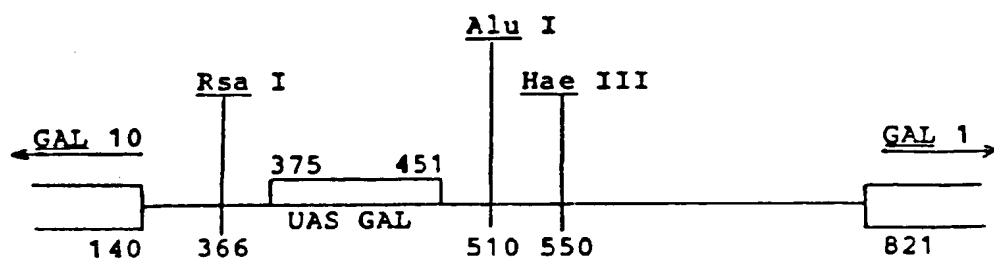
9

Fig.1.



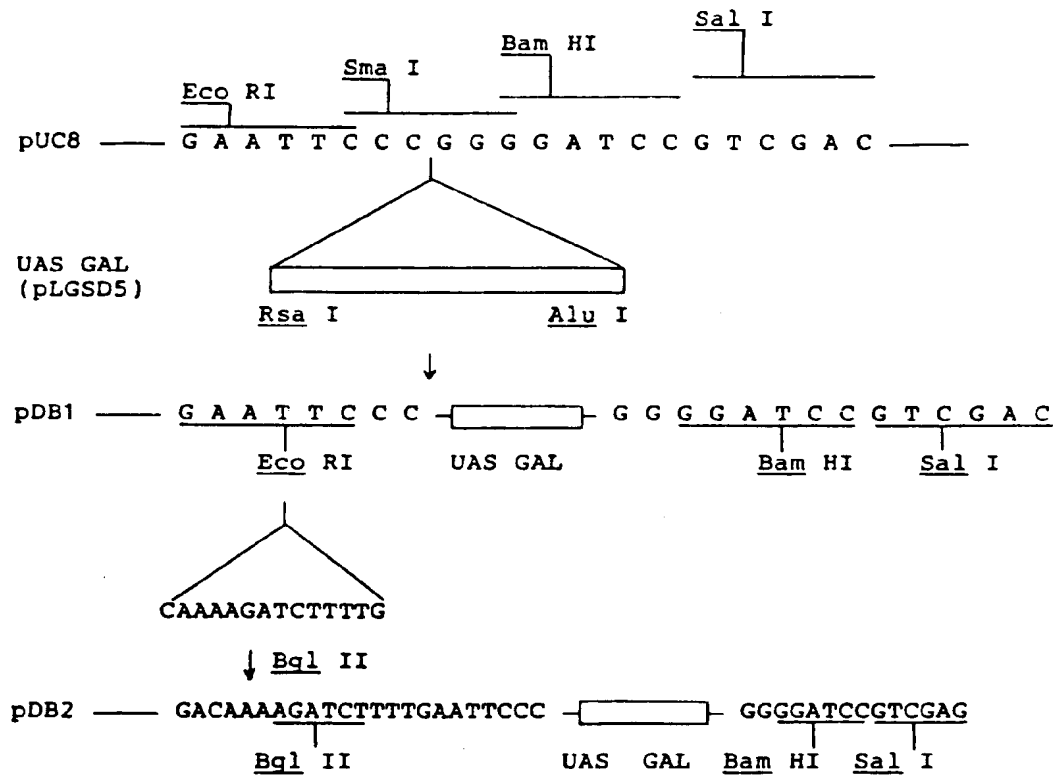
*Fig. 2.*

Diagrammatic representation of the divergent GAL1-GAL10 promoter region of *S. cerevisiae*



*Fig.3.*

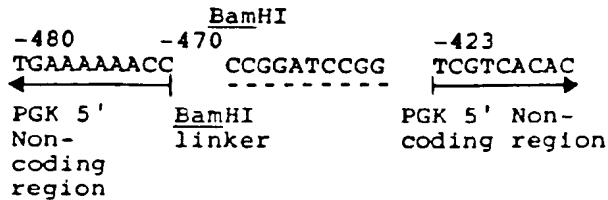
Construction of UAS GAL plasmids pDB1 and pDB2



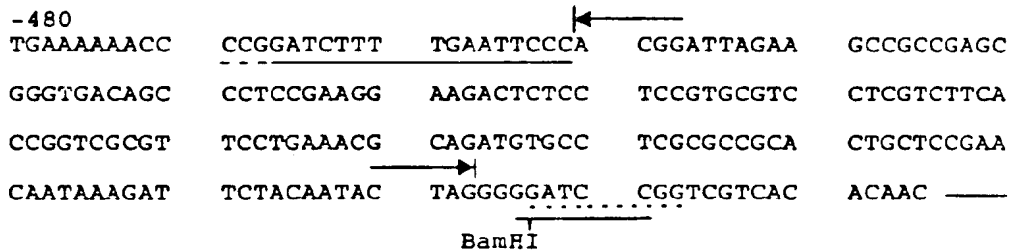
*Fig.4.*Bal 31 deletion series of pDB4

<u>Deletion/ Plasmid</u>	<u>-20</u>	<u>-10</u>	<u>-1</u>	
pDB4	TTTACAACAA	ATATAAAACA	ATGTCTTTAT	CTTCA
-8 (pKV47, pKV49, pKV50)	TTTACAACAA	<u>ATACAAAAGA</u>	<u>TCTTTTG</u>	
+4 (pKV51, pKV61, pKV62)	TTTACAACAA	ATATAAAACA	<u>ATGTCAAAAG</u>	<u>ATCTTTTG</u>
+5 (pKV52, pKV63, pKV64)	TTTACAACAA	ATATAAAACA	<u>ATGTCCAAAA</u>	<u>GATCTTTTG</u>
+6 (pKV53, pKV65, pKV66)	TTTACAACAA	ATATAAAACA	<u>ATGTCTCAAA</u>	<u>AGATCTTTTG</u>

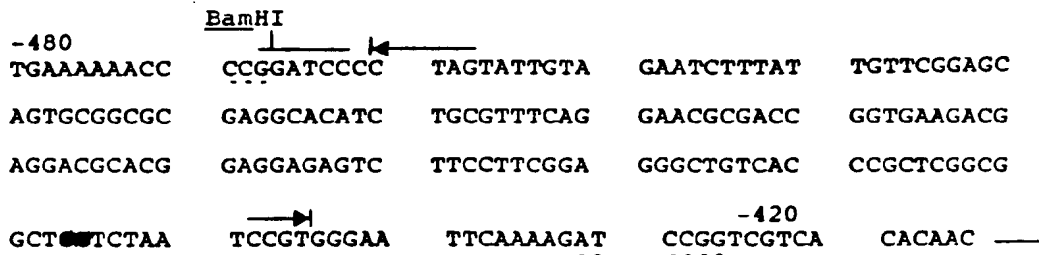
The underlined region represents the DNA sequence of the BglII synthetic oligonucleotide linker.

*Fig. 5.*5' Non-Coding Region of pDB4 and the Hybrid PGK-GAL-UAS Promoter ConstructspDB4

Plasmids: pKV43, pKV49, pKV61, pKV63, pKV65.



Plasmids: pKV44, pKV 50, pKV62, pKV64, pKV66.

Legend to Figure 5

Numbers indicate the relative position in the 5' non-coding region of the PGK gene.

The region bounded by arrows represents the GAL-UAS DNA sequence (Johnston & Davis, 1984).

The underlined region indicates the DNA sequence corresponding to the synthetic Bgl II oligonucleotide linker. The DNA sequence underlined by the broken line indicates the nucleotides derived from the synthetic BamHI oligonucleotide linker in pDB4.

Fig. 6

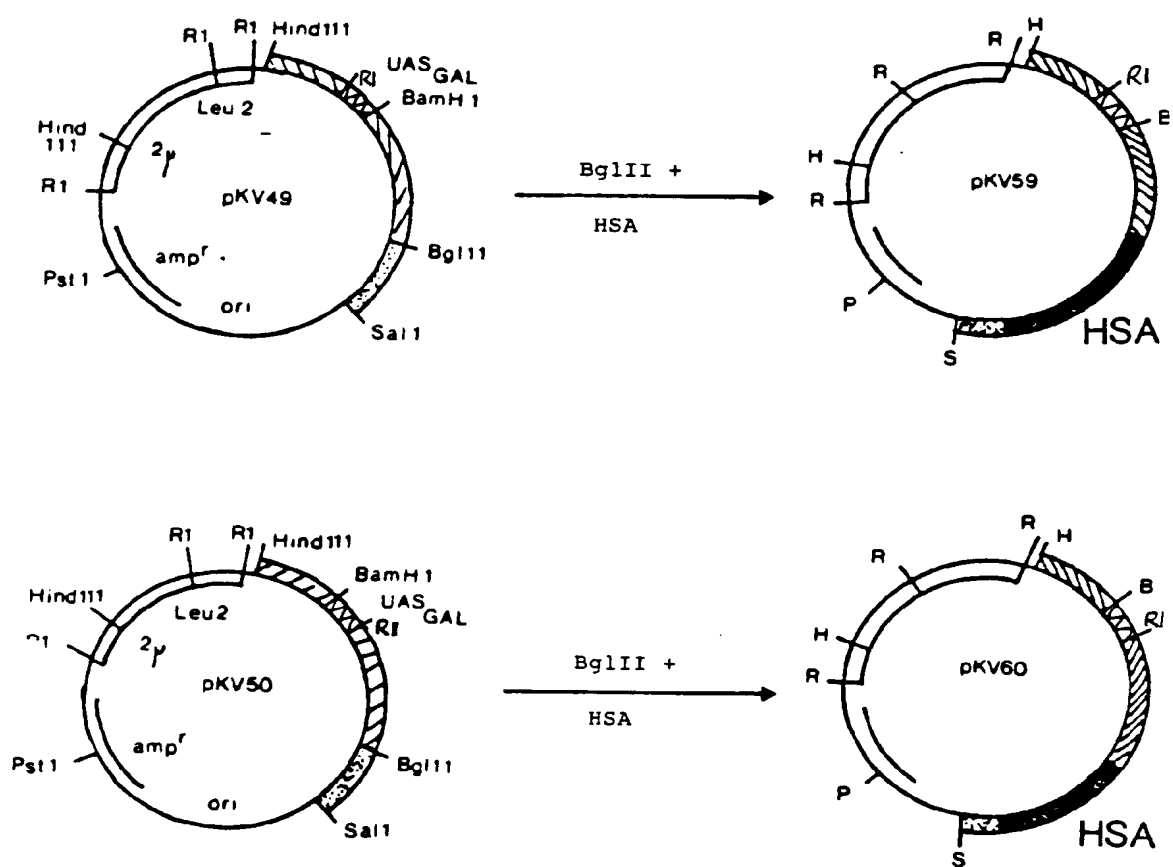


Fig. 7 DNA sequence at the 5' junction of the HSA gene and the  
PGK-GAL hybrid promoter in plasmids pKV59 and pKV60

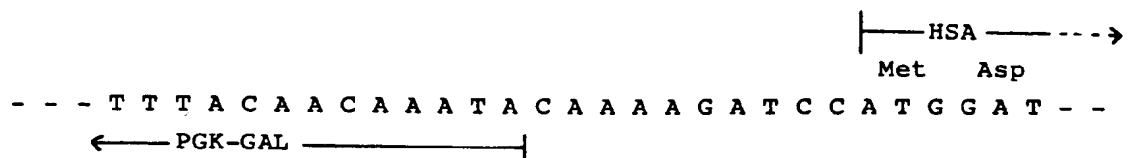
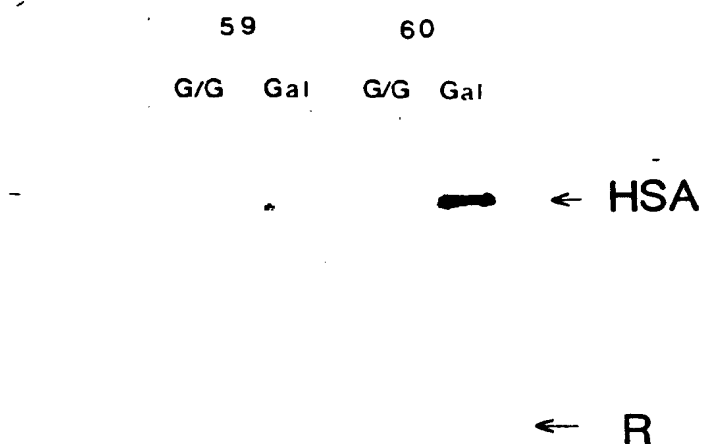


Fig. 8 Autoradiograph of Northern blotted total yeast RNA isolated from DBY745(pKV59) and DBY745 (pKV60)



RNA was probed with HSA and ribosomal (R) specific DNA probes, the latter being an internal loading standard. RNA was prepared from galactose grown yeast (Gal) and galactose plus glucose grown yeast (G/G).